Exogenous Cholesterol Transport in Rabbit Plasma Lipoproteins

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1. The appearance of exogenous cholesterol in free cholesterol and ester cholesterol of plasma chylomicra, very-low-density (VLD), low-density (LD) and high-density (HD) lipoproteins was studied in unanaesthetized rabbits after ingestion of a meal containing 5% fat and 0.08% [^3H]cholesterol. 2. The specific radioactivity of ester cholesterol of VLD lipoproteins reached the highest value of any lipoprotein fraction and for each lipoprotein it increased at a faster rate and reached a higher maximum than that of free cholesterol; the maximum in VLD lipoproteins occurred later than in chylomicra. 3. The pattern of appearance of exogenous cholesterol in chylomicra and VLD lipoproteins of plasma was similar to the pattern previously observed in lymph. The specific radioactivity of ester cholesterol in plasma VLD lipoproteins was higher than that in chylomicra in spite of a larger pool size and dilution of cholesteryl esters from VLD lipoproteins produced by the liver. These results support the concept that during absorption the major portion of exogenous cholesterol is transported in VLD lipoproteins as ester cholesterol. 4. The specific radioactivity of ester cholesterol of chylomicra and VLD lipoproteins increased at a faster rate than that of LD and HD lipoproteins. However, the rate of increase and the absolute values of the specific radioactivity in LD and HD lipoproteins were identical. Since cholesteryl esters are thought not to exchange between lipoproteins, this observation supports the hypothesis that a result of VLD lipoprotein and chylomicron metabolism is the formation of LD and HD lipoproteins. 5. Results *in vivo* showed that the free cholesterol of individual plasma lipoproteins does not equilibrate within a period of 24h.

Many reports (Miettinen, 1957; Vannini & Barbara, 1958; Grundy & O'Neal, 1963; Chung *et al.*, 1969) show that the distribution of cholesterol among plasma lipoproteins in normal rabbits is similar to the distribution in normal humans (Fredrickson & Levy, 1972) although absolute concentrations in humans are higher. The present report provides further documentation of this similarity. Rabbits fed on diets high in cholesterol have long been known to exhibit increased plasma cholesterol concentrations with the subsequent development of atherosclerosis. These findings establish the rabbit as a valuable experimental model for study of the relationship between exogenous cholesterol metabolism and plasma lipoprotein metabolism. In spite of this, most of the work relating these two areas of lipid metabolism has been carried out in other species.

In a previous study (Rudel *et al.*, 1972) rabbits were fed on a diet with a low cholesterol–moderate fat content and sequential lymph samples were obtained between 3 and 24h post-prandially. We found that

labelled exogenous cholesterol was preferentially transported as cholesteryl ester in VLD lipoproteins.† At all time-intervals VLD lipoproteins had a higher concentration of exogenous cholesterol than did chylomicra and together these lipoproteins accounted for over 90% of the exogenous cholesterol in lymph. It was decided to follow the subsequent fate of the exogenous cholesterol of these lipoproteins after they entered the circulation.

Materials and Methods

New Zealand white rabbits of both sexes weighing between 3–4kg were used in these studies. Animals were trained to eat meals as described by Rudel *et al.* (1972). Diet consisted of Rockland rabbit chow (Teklad, Monmouth, Ill., U.S.A.) to which was added 5% (w/w) stripped lard (Eastman Kodak Company, Rochester, N.Y., U.S.A.) and 0.08% cholesterol (Nutritional Biochemicals Corp., Cleveland, Ohio,

† Abbreviations: VLD lipoproteins, very-low-density lipoproteins; LD lipoproteins, low-density lipoproteins; HD lipoproteins, high-density lipoproteins.

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U.S.A.). On the day of an experiment animals received their normal morning meal, to which had been added $15\mu\text{Ci}$ ($3.3 \times 10^6 \text{ d.p.m./}\mu\text{g}$) of [$7\alpha\text{-}^3\text{H}$]cholesterol (New England Nuclear Corp., Boston, Mass., U.S.A.). Labelled cholesterol was purified on t.l.c. before use (de Vries & Jurriens, 1963). The animals remained in their cages throughout the experiment and had access to water *ad libitum*.

Sample collection and subsequent fractionation

Blood was obtained from each animal at four time-intervals after the ingestion of a meal. Each sample of blood (10–12 ml) was collected from the marginal ear vein of unanaesthetized rabbits into tubes which contained EDTA (1 mg/ml) to inhibit clotting and 5,5'-dithiobis-(2-nitrobenzoic acid) (Sigma Chemical Co., St. Louis, Mo., U.S.A.) as an inhibitor of plasma phosphatidylcholine-cholesterol acyltransferase (Gidez *et al.*, 1967). Plasma was separated from erythrocytes by centrifugation at 1000g for 15 min at room temperature immediately after collection. Chylomicra were isolated without delay after layering plasma under buffered solution of *d* 1.006 (Scanu & Granda, 1966) followed by centrifugation at 20000g for 30 min ($1 \times 10^6 g_{av.}\text{-min}$) at 15°C in a Sorvall RC-2 centrifuge (type SS-34 head). Tubes were cut with a tube slicer and the top turbid fraction was then removed. The remaining plasma lipoproteins (VLD, LD and HD lipoproteins) were isolated by sequential ultracentrifugation in an L2-65B ultracentrifuge (Beckman Instruments, Palo Alto, Calif., U.S.A.) with an SW-40 rotor at 15°C as described by Havel *et al.* (1955) and modified as described by Radding & Steinberg (1960). Isolated lipoprotein fractions were stored at 4°C until analysed. (Analyses were completed within 2 weeks of collection.) VLD-, LD- and HD-lipoprotein fractions isolated in this way were essentially free of other lipoprotein classes as seen after lipid staining of agarose gels after electrophoresis (Plate 2) and they were not subjected to further centrifugation. The chylomicron fraction contained several lipid-staining components, which could not be removed by repeated centrifugation.

Agarose electrophoresis

Agarose electrophoresis (Noble, 1968) was used to characterize both plasma samples and isolated plasma lipoprotein fractions. Electrophoresis strips were stained overnight at 40°C in a 60% (v/v) ethanol solution saturated with Oil Red O.

A simple, quantitative method for determining the distribution of plasma cholesterol among lipoproteins after densitometric scanning of agarose-electrophoresis strips was developed by using a Beckman Analytrol densitometer and is similar to a method described by Lindgren *et al.* (1972). Purified lipo-

protein preparations, containing known cholesterol concentrations (determined as below), were mixed in known proportions to approximate original plasma concentrations. The lipoprotein mixture was then subjected to agarose electrophoresis and the electrophoretic patterns that resulted were scanned in the densitometer. Conversion factors were obtained which related areas observed to the proportion of cholesterol in each lipoprotein. Factors for relating percentage area to cholesterol content of each of the lipoprotein fractions were determined in triplicate on two separate mixtures and were 0.46 for VLD lipoprotein, 1.20 for LD lipoprotein and 0.91 for HD lipoprotein. (Chylomicra were present in too small a quantity to be determined by this technique.) When the cholesterol distribution among lipoproteins of plasma samples was determined, the absolute values for each fraction were based on a determination of total plasma cholesterol. The validity of this technique was checked, in a separate group of determinations of plasma lipoprotein cholesterol distribution, by the method of Fredrickson *et al.* (1967). This method employs an initial centrifugation at *d* 1.006 and a heparin- Mn^{2+} precipitation, after which the cholesterol distribution is determined. Both methods gave identical results.

Extraction and analysis of samples

Lipoprotein solutions were extracted in chloroform-methanol (2:1, v/v) by the method of Bragdon (1960) with a solvent/solute ratio of 25:1. Samples of the chloroform layer were evaporated under N_2 , transferred to t.l.c. plates, and the lipids separated as described by Rudel *et al.* (1972). Cholesteryl ester from the t.l.c. plate was saponified. Free cholesterol and ester cholesterol were determined by using [^{14}C]acetyldigtonin precipitation (Morris, 1965), as was total cholesterol determined directly after saponification. After double-radioisotope counting, d.p.m. and specific radioactivity were calculated by computer by using the channels-ratio method for quench correction (Peng, 1970).

Statistics

Statistical analyses of differences in the specific radioactivities of ester cholesterol and free cholesterol were performed by using the paired *t* test, or where a non-parametric method was necessary, the signed rank test for paired samples was used (Snedecor & Cochran, 1967).

Results

Agarose electrophoresis and densitometry of sequential plasma samples were performed. Plate 1 shows a typical series of plasma samples for one

EXPLANATION OF PLATE I

Agarose-electrophoresis patterns of plasma samples sequentially collected from a single rabbit after a meal

Strip 1 shows plasma collected 3 h after a meal. Strip 2 shows plasma collected 6 h after a meal. Strip 3 shows plasma collected 12 h after a meal. Strip 4 shows plasma collected 24 h after a meal.

EXPLANATION OF PLATE 2

Agarose-electrophoresis patterns of lipoprotein classes obtained after ultracentrifugal fractionation of a typical plasma sample

Strip 1 shows the whole plasma before fractionation. Strip 2 shows plasma after a $1 \times 10^6 g$ -min centrifugation; the chylomicra isolated in the top layer after this centrifugation are shown in strip 3. The plasma was then re-centrifuged for $120 \times 10^6 g$ -min at $d1.006$ and the VLD lipoproteins isolated from the top layer after this centrifugation are shown in strip 4. After increasing the density to $d1.063$, the plasma was re-centrifuged and the LD lipoproteins isolated from the top fraction are shown in strip 5. The plasma density was then raised to $d1.21$, and the HD lipoproteins isolated from the top fraction are shown in strip 6.

animal. During absorption of the meal, increased plasma chylomicra and VLD lipoproteins are present. At 12h no chylomicra are seen although an increase in VLD lipoproteins is still evident compared with the 24h-starved sample. No differences in the concentration of LD and HD lipoproteins were clearly demonstrated by this technique.

The percentage distribution of cholesterol among three plasma lipoprotein fractions (VLD, LD and HD lipoproteins) was determined by densitometry. Although individual rabbits showed a change in the pool size of VLD lipoproteins with time after the meal (Plate 1), the average values for all lipoprotein classes for six animals showed no statistically significant variation in cholesterol distribution with time. The mean values $\pm$ S.E.M. for the percentage of total plasma cholesterol found in VLD, LD and HD lipoproteins were 8.79 ± 0.75 , 60.09 ± 2.47 and 30.77 ± 2.33 , respectively.

Lipoproteins isolated by ultracentrifugation from sequential plasma samples were checked for purity by using agarose electrophoresis and lipid staining (Plate 2). Strip 2 shows plasma after chylomicra were removed by centrifugation. Electrophoresis of chylomicra (strip 3) showed detectable Oil Red O-staining material in the regions of other lipoproteins. Electrophoresis of LD, VLD and HD lipoproteins showed the presence of a single major band for each fraction although small amounts of material in the LD-lipoprotein region were usually found in the HD-lipoprotein fraction.

Fig. 1 shows the mean values for the specific radioactivity of free cholesterol and ester cholesterol of plasma lipoproteins isolated from four sequential plasma samples obtained from eight rabbits, three animals at 3, 6, 12 and 24h, and five animals at 3, 9, 15 and 24h. In every plasma lipoprotein, the specific radioactivity of the ester cholesterol increased at a more rapid rate than that of free cholesterol and reached a higher maximum. The difference between specific radioactivity of ester cholesterol and free cholesterol was highly significant ($P < 0.001$); in all of the 104 paired observations for plasma lipoproteins, the specific radioactivity of the ester cholesterol exceeded that of the free cholesterol.

To study the pattern of change in specific radioactivity of ester cholesterol among individual lipoprotein classes, irrespective of differences in absolute values between animals, values for each lipoprotein fraction in each animal were expressed in terms of the 24h value for VLD lipoprotein = 100%. The results in Fig. 2 show the relationships between the values of the separate lipoprotein classes. The value for VLD lipoproteins increased rapidly and reached 160% at the maximum (12h). The value for chylomicra also increased rapidly and reached a maximum of 120% at about 7.5h. The values for LD and HD lipoproteins increased at identical rates, attained plateau values of

Fig. 1. Specific radioactivities of free cholesterol and ester cholesterol of lipoprotein classes in plasma collected sequentially from rabbits after a meal containing [3 H]cholesterol

The mean specific radioactivity for ester cholesterol (●, eight animals) and for free cholesterol (○, six animals) is shown. For purposes of statistical evaluation, samples collected at 6 and 9h and at 12 and 15h have been averaged together and plotted at 7.5h and 13.5h, respectively. (a) Chylomicra; (b) VLD lipoproteins; (c) LD lipoproteins; (d) HD lipoproteins.

100% at about 13.5h and remained constant during the next 10h. The results of statistical analyses of differences between paired observations of the specific radioactivity of cholesteryl esters in the various lipoproteins are shown in Table 1. The specific radioactivity in VLD lipoprotein was significantly higher than in chylomicra at the 7.5, 13.5 and 24h time-intervals and was significantly higher than in LD and HD lipoproteins at 3, 7.5 and 13.5h. The specific radioactivity in chylomicra was significantly higher than in LD and HD lipoproteins at the 3 and 7.5h time-intervals.

The ratios between the specific radioactivities of free cholesterol of the individual lipoprotein classes are shown in Fig. 3. Statistical analyses of differences are shown in Table 2. The specific radioactivity in VLD lipoproteins was higher than that in chylomicra at all times studied; the difference was statistically significant at 7.5, 13.5 and 24h after feeding (Table 2). Thus the chylomicra/VLD lipoproteins ratio was always less than 1 (Fig. 3). The ratio of LD/VLD lipoproteins increased throughout the course of the study and exceeded 1.0 at 24h. The difference between free cholesterol specific radioactivities of LD and VLD lipoproteins at 24h just failed to reach the 5% significance level. The specific radioactivity in HD

lipoproteins was low throughout the study and was significantly lower than in LD and VLD lipoproteins at all times studied.

Discussion

A study of the transport of exogenous (dietary) cholesterol in plasma was carried out in rabbits fed on low cholesterol-moderate fat diets by following

the time-course of appearance of exogenous [³H]-cholesterol in plasma lipoproteins after a meal. Rudel *et al.* (1972) demonstrated that during absorption exogenous cholesterol is transported from the intestinal mucosal cell via the thoracic lymph duct principally in the form of esterified cholesterol of VLD lipoproteins and chylomicra. The VLD lipoproteins were found to transport significantly more of the exogenous cholesterol than did chylomicra. In the present work, exogenous cholesterol was found to be present in plasma VLD lipoproteins and chylomicra principally as esterified cholesterol; 70% of total cholesterol is esterified in rabbit plasma chylomicra and VLD lipoproteins (Rudel, 1970). The peak in specific radioactivity of ester cholesterol in plasma VLD lipoproteins occurred at approx. 13.5 h after the meal and was later than the corresponding peak in plasma chylomicra. This temporal relationship was also found in lymph chylomicra and VLD lipoproteins (Rudel *et al.*, 1972). The specific radioactivity of ester cholesterol in plasma VLD lipoproteins reached the highest maximum of any plasma-lipoprotein fraction, including chylomicra. Thus the present results provide additional evidence that during absorption VLD lipoproteins are the primary transport vehicle of exogenous cholesterol as esterified cholesterol.

It is believed that the studies presented in this paper are unique in that the animals utilized were maintained in as completely a physiological state as possible. No anaesthesia, surgery or other manipulation was used to administer the [³H]cholesterol dose to the animals. Rather the dose was completely consumed within 30 min as part of a regular 45 g meal. The total amount of blood collected from each animal over the 24 h period of these experiments was 48 ml, which would represent about 18% of the total blood volume if taken all at one time. However, the effects of blood loss were minimized by collecting samples over a 24 h period; thus although a small decrease in total pool size may have occurred the relationships between plasma lipoproteins probably were unaltered. It is noteworthy that the time-course of exo-

Fig. 2. Pattern of change of specific radioactivity of ester cholesterol in plasma lipoproteins of rabbits fed on a meal containing [³H]cholesterol

Each point represents the mean $\pm$ S.E.M. for eight animals. All values are expressed in terms of the 24 h value for VLD lipoproteins = 100%, as described in the text. ●, Chylomicra; ○, VLD lipoproteins; □, LD lipoproteins; △, HD lipoproteins.

Table 1. Significance of differences in the specific radioactivity of ester cholesterol of plasma lipoproteins in rabbits fed on a meal containing 5% fat

The meal contained 0.08% of [³H]cholesterol. Analytical techniques are described in the text. Calculations were made by using the paired *t* test except where indicated, *, when the signed rank test was used (see the text). N.S., not significant.

Time after meal (h)	VLD lipoproteins versus chylomicra	VLD lipoproteins versus LD lipoproteins	VLD lipoproteins versus HD lipoproteins	Chylomicra versus LD lipoproteins	Chylomicra versus HD lipoproteins	LD lipoproteins versus HD lipoproteins
3	N.S.	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.005	<i>P</i> < 0.005	N.S.
7.5	<i>P</i> < 0.05*	<i>P</i> < 0.01*	<i>P</i> < 0.01*	<i>P</i> < 0.05	<i>P</i> < 0.05	N.S.
13.5	<i>P</i> < 0.01*	<i>P</i> < 0.01*	<i>P</i> < 0.05*	N.S.	N.S.	N.S.
24	<i>P</i> < 0.005	N.S.	N.S.	N.S.	N.S.	N.S.

genous cholesterol appearance in the present experiments agrees closely with experiments in which thoracic lymph-duct cannulations were performed (Rudel *et al.*, 1972).

Both the rate of increase and the maximum value obtained for the specific radioactivity of ester chol-

Fig. 3. Ratios of specific radioactivities of free cholesterol in lipoproteins isolated from sequentially collected plasma samples of rabbits fed on a meal containing [³H]cholesterol

The mean $\pm$ S.E.M. of determinations from six animals are shown. Ratio of specific radioactivities of free cholesterol are shown for LD lipoproteins/HD lipoproteins (●); for LD lipoproteins/VLD lipoproteins (□); for chylomicra/VLD lipoproteins (○); for HD lipoproteins/VLD lipoproteins (Δ).

esterol of plasma LD and HD lipoproteins exceeded that of the respective free cholesterol (Fig. 1). Therefore it is likely that exogenous cholesteryl ester of chylomicra and VLD lipoproteins appeared in LD and HD lipoproteins without uptake, hydrolysis and re-esterification by the liver, as Quarfordt & Goodman (1967) suggested occurred in the rat. Attempts to show significant exchange of lipoprotein cholesteryl ester between lipoproteins *in vitro* have not been successful in other species (Goodman, 1962; Minari & Zilversmit, 1963; Roheim *et al.*, 1963) and we have not observed any exchange with rabbit lipoproteins (L. L. Rudel & M. D. Morris, unpublished work). (In this sense 'exchange' is defined as a reversible process in which no net transfer occurs, in contrast with a net movement between lipoproteins as may occur during lipoprotein metabolism.) Thus the higher specific radioactivity of ester cholesterol compared with that of free cholesterol, observed for plasma LD and HD lipoproteins, is probably not due to exchange of exogenous cholesteryl ester between VLD lipoproteins or chylomicra and LD and HD lipoproteins. The observation that the specific radioactivity of ester cholesterol increased much more rapidly than that of free cholesterol in all plasma lipoproteins excludes the possibility that exogenous free cholesterol in plasma was the major precursor of plasma lipoprotein [³H]cholesteryl ester, as would occur in the plasma phosphatidylcholine-cholesterol acyltransferase reaction (Glomset, 1968).

An alternative explanation is that in the rabbit exogenous cholesteryl esters are translocated into plasma LD and HD lipoproteins during formation of these lipoproteins by intravascular metabolism of the VLD lipoproteins and chylomicra after they enter plasma. The exact mechanism by which this happens is not clear at this time. However, it is presumed that such a transformation would occur as a result of, or shortly after, lipoprotein lipase-catalysed hydrolysis and removal of triglyceride from the exogenous lipoproteins. From our results it seems that the appearance of exogenous ester cholesterol in LD

Table 2. Statistical significance of differences in the specific radioactivity of free cholesterol of plasma lipoproteins in rabbits fed on a meal containing 5% fat

The meal contained 0.08% of [³H]cholesterol. Analytical techniques are described in the text. Calculations were made by using the paired *t* test except where indicated, *, when the signed rank test was used (see the text). N.S., not significant.

Time after meal (h)	VLD lipoproteins versus chylomicra	VLD lipoproteins versus LD lipoproteins	VLD lipoproteins versus HD lipoproteins	Chylomicra versus LD lipoproteins	Chylomicra versus HD lipoproteins	LD lipoproteins versus HD lipoproteins
3	N.S.	<i>P</i> < 0.01	<i>P</i> < 0.001	<i>P</i> < 0.025	<i>P</i> < 0.001	<i>P</i> < 0.001
7.5	<i>P</i> < 0.025	<i>P</i> < 0.05	<i>P</i> < 0.01	N.S.	N.S.	<i>P</i> < 0.005
13.5	<i>P</i> < 0.025	N.S.	<i>P</i> < 0.05	<i>P</i> < 0.025	N.S.	<i>P</i> < 0.025
24	<i>P</i> < 0.025	N.S.	<i>P</i> < 0.05*	<i>P</i> < 0.025	N.S.	<i>P</i> < 0.005

and HD lipoproteins occurs as a result of a single metabolic event since the rate of increase and the absolute values for the specific radioactivity of ester cholesterol of LD and HD lipoproteins were the same throughout the study (Fig. 2). A precedent for this interpretation of our results comes from studies in other laboratories in which lipoprotein lipase-mediated metabolism of VLD lipoproteins gives rise to a shift of apoproteins (LaRosa *et al.*, 1971; Windmueller *et al.*, 1971; Bilheimer *et al.*, 1972) and cholesterol (LaRosa *et al.*, 1971) from VLD lipoproteins to plasma LD and HD lipoproteins.

The fraction of plasma VLD lipoprotein ester cholesterol that originates from intestinal lymph can be calculated when previous data (Rudel *et al.*, 1972) and the present results are combined. All animals were fed on diets containing the same amount of cholesterol and fat. The maximum rate of total cholesterol appearance in lymph VLD lipoproteins was 5 mg/h and this was about 60% esterified; thus 3 mg of ester cholesterol appearance/h was observed for VLD lipoproteins. The maximum for the specific radioactivity of ester cholesterol in lymph VLD lipoproteins was 1×10^6 d.p.m./mg and was observed at the same time as the maximum rate of appearance. Specific radioactivity of dietary cholesterol was 1.8×10^6 d.p.m./mg for the lymph studies. In the present studies the observed maximum specific radioactivity of ester cholesterol in plasma VLD lipoproteins was 1×10^5 d.p.m./mg. Specific radioactivity of dietary cholesterol was 0.9×10^6 d.p.m./mg, or half that of the earlier study. For calculation purposes, the maximum specific radioactivity of the ester cholesterol of VLD lipoproteins was corrected to 2×10^5 d.p.m./mg since dietary cholesterol specific radioactivities differed 2-fold. Thus the specific radioactivity ratio for lymph VLD/plasma VLD lipoproteins was 5:1 and the rate of ester cholesterol input from endogenous sources was calculated to be 12 mg/h. The total calculated VLD lipoprotein ester cholesterol turnover was 15 mg/h. On this basis, during the maximal rate of exogenous cholesterol absorption, the input of plasma VLD lipoprotein ester cholesterol from the liver appears to be 4 times faster than the rate of input from VLD lipoproteins of the lymph.

The plasma VLD lipoproteins contain about 10% of the plasma cholesterol. Assuming a plasma volume of 4% of body weight in 3.5 kg rabbits with an average cholesterol concentration of 75 mg/dl, which was observed for these animals (Rudel, 1970), the VLD lipoprotein pool size of total cholesterol is about 10 mg. Since about 70% of total cholesterol in the plasma VLD lipoproteins is ester cholesterol (Rudel, 1970) the ester cholesterol pool size for plasma VLD lipoprotein ester cholesterol is about 7 mg. It can be calculated from these values that the turnover time in the rabbit for plasma VLD lipoprotein ester chole-

sterol is 28 min and the half-time is 19 min. This value is similar to the half-time calculated after reinjection of labelled VLD lipoproteins in rats (Ockner *et al.*, 1969) and is comparable with the value of 13 min obtained in starved rabbits for VLD lipoprotein triglyceride by Havel *et al.* (1962). Since the rate of turnover of VLD lipoprotein ester cholesterol and VLD lipoprotein triglyceride is similar, and since we found that exogenous ester cholesterol of plasma VLD lipoproteins does not undergo significant exchange, our results suggest that turnover of VLD lipoprotein ester cholesterol in the rabbit accurately reflects turnover of VLD lipoproteins *per se*.

Since many of the differences between the values of the specific radioactivity of free cholesterol in the various lipoproteins were highly significant, these studies demonstrate that all free cholesterol of plasma lipoproteins does not equilibrate *in vivo*. We have no evidence to indicate whether a rapidly increasing specific radioactivity in free cholesterol of lipoprotein indicates less dilution of exogenous cholesterol by rapid exchange, or whether the rate of entry of exogenous free cholesterol into this pool is higher, independent of random exchange rates. Based on the hypothesis of Gurd (1960) and work of Zilversmit (1968), in which free cholesterol exchange is not dependent on enzyme activity, it would appear that the free cholesterol in the lipoprotein outer surface is in a different protein-phospholipid environment in each lipoprotein or that all free cholesterol is not in the outer surface and available for exchange.

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